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Interleukin-15 Increases Vaccine Efficacy through a Mechanism Linked to Dendritic Cell Maturation and Enhanced Antibody Titers▽

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Interleukin-15 (IL-15) is generally considered to sustain T-cell memory and to be a growth factor for natural killer cells. Previous data from our laboratory demonstrated that IL-15 is also an important factor for developing human dendritic cells. For this study, we investigated the effects of IL-15 on antibody responses in mice to a recombinant staphylococcal enterotoxin B (SEB) vaccine (STEBVax) in a preclinical model of toxic shock syndrome induced by SEB. We observed that mouse spleen cells treated with IL-15 in ex vivo culture gained a dendritic cell-like phenotype. Administration of IL-15 to mice also resulted in an increased number of mature CD11c$^+$ dendritic cells in mouse spleens. A significant, IL-15 dose-dependent increase in antigen-specific antibody was observed after coadministration with the vaccine and an aluminum-based adjuvant (alhydrogel). Furthermore, the coadministration of IL-15 with STEBVax and alhydrogel also protected mice from lethal toxic shock above the levels that obtained without IL-15. Thus, the vaccine response enhanced by IL-15 appears to be mediated by mature dendritic cells and results in prevalent seroconversion to Th2-dependent antibodies. This suggests a potential use of IL-15 as an adjuvant for antibody-dependent responses to vaccines.

Interleukin-15 (IL-15) plays a pivotal role in the regulation of innate and adaptive immunity because of its effect on both lymph- and bone marrow-derived cells of the immune system (5, 29, 41). Most reports indicate an immune modulator function for IL-15. For example, exogenous administration of recombinant IL-15 before infection with *Salmonella enterica*, *Toxoplasma gondii*, *Plasmodium falciparum*, or *Cryptococcus neoformans* improves host defense against, and clearance of, the invading organisms (10, 12, 18, 22). Systemic administration of IL-15 to mice augments the antigen-specific primary CD8$^+$ T-cell response after vaccination with peptide-pulsed dendritic cells (DC) (27). In vivo expression of IL-15 in transgenic mice after the inoculation of *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) 24 weeks earlier elicited resistance against airborne infection with *Mycobacterium tuberculosis*, accompanied by an increased CD8$^+$ T-cell response (39). In addition, IL-15 has been shown to have a major impact on natural killer cells and natural killer T cells, further influencing immune regulation (19, 24). Other reports also indicate that IL-15 is crucial for the maintenance of CD8$^+$/high-expression CD44 memory phenotype T cells, natural killer cells, and CD8$^+$ memory T cells (15, 31, 40). Thus, accumulating evidence suggests that the effects of IL-15 are directed toward cellular immunity and result in the modulation of survival and the cycling of established memory cell populations (7, 13, 33).

The IL-15 message has been found in a variety of cell types (9, 21), while IL-15 protein is secreted at low levels. The highest expression levels are found in monocytic cells, including DC (7). It has been proposed that DC-derived IL-15 serves as a positive-feedback signal for DC activation and maturation to promote DC–T-cell interactions in the context of T-cell-dependent specific immune responses (28). Further, IL-15 was reported to be involved in an autocrine loop that was resistant to the apoptosis that accompanies the maturation process of DC in vitro (7). It was also reported that the transduction of DC with the IL-15 gene markedly stimulates DC function and protects them from tumor-induced apoptosis (35). Recent reports suggest that IL-15 is produced by follicular DC (FDC), captured by IL-15 receptor alpha of FDC/HK, and *trans*-presented to germinal-center (GC) B cells and that, as a result of the *trans*-presentation, it supports proliferation and immune modulation (25, 30). These findings indicate that IL-15 may also influence B cells and DC (4, 16, 36). Whether or not IL-15 contributes to the functional activity of DC in vivo for inducing antigen-specific humoral immunity was not previously assessed. STEBVax is a recombinant staphylococcal enterotoxin B (SEB) vaccine used for the prevention of toxic shock syndrome and certain infections caused by *Staphylococcus aureus*. Earlier work from our laboratory showed that IL-15-induced human mature DC support STEBVax-specific T-cell responses ex vivo (29). For this murine study, we investigated the effects of IL-15 on DC maturation as well as the ability of IL-15 to serve as a DC activator and thereby augment antibody responses to STEBVax. The results indicate that IL-15 enhanced the protective antibody response and that DC maturation induced by IL-15 was partially responsible for the enhancement.

MATERIALS AND METHODS

SEB vaccine and reagents. The recombinant SEB vaccine B899445C (STEBVax), previously reported, was prepared under Good Manufacturing Practice (39b) conditions, as described previously (6, 38). Mouse IL-15 was purchased from

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14. ABSTRACT
Interleukin-15 (IL-15) is generally considered to be a growth factor for natural killer cells and for sustaining T-cell memory. Previous data from our laboratory demonstrated that IL-15 is also an important factor for developing human dendritic cells. In this study, we investigated the effect of IL-15 on antibody responses to a recombinant staphylococcal enterotoxin B (SEB) vaccine (STEBVax), in a pre-clinical model of toxic-shock syndrome induced by SEB. We observed that mouse spleen cells treated with IL-15 in ex vivo culture gained a dendritic cell-like phenotype. Administration of IL-15 to mice also resulted in an increased number of mature CD11c+ dendritic cells in mouse spleens. A significant, IL-15 dose-dependent increase in antigen-specific antibody was observed after co-administration with vaccine and an aluminum-based adjuvant (alhydrogel). Furthermore, the co-administration of IL-15 with STEBVax and alhydrogel also protected mice from lethal toxic shock above the levels that obtained without IL-15. Thus, the vaccine response enhanced by IL-15 appears to be mediated by mature dendritic cells, and results in prevalent sero-conversion to Th2-dependent antibodies. This suggests a potential use of IL-15 as an adjuvant for antibody-dependent responses to vaccines.

15. SUBJECT TERMS
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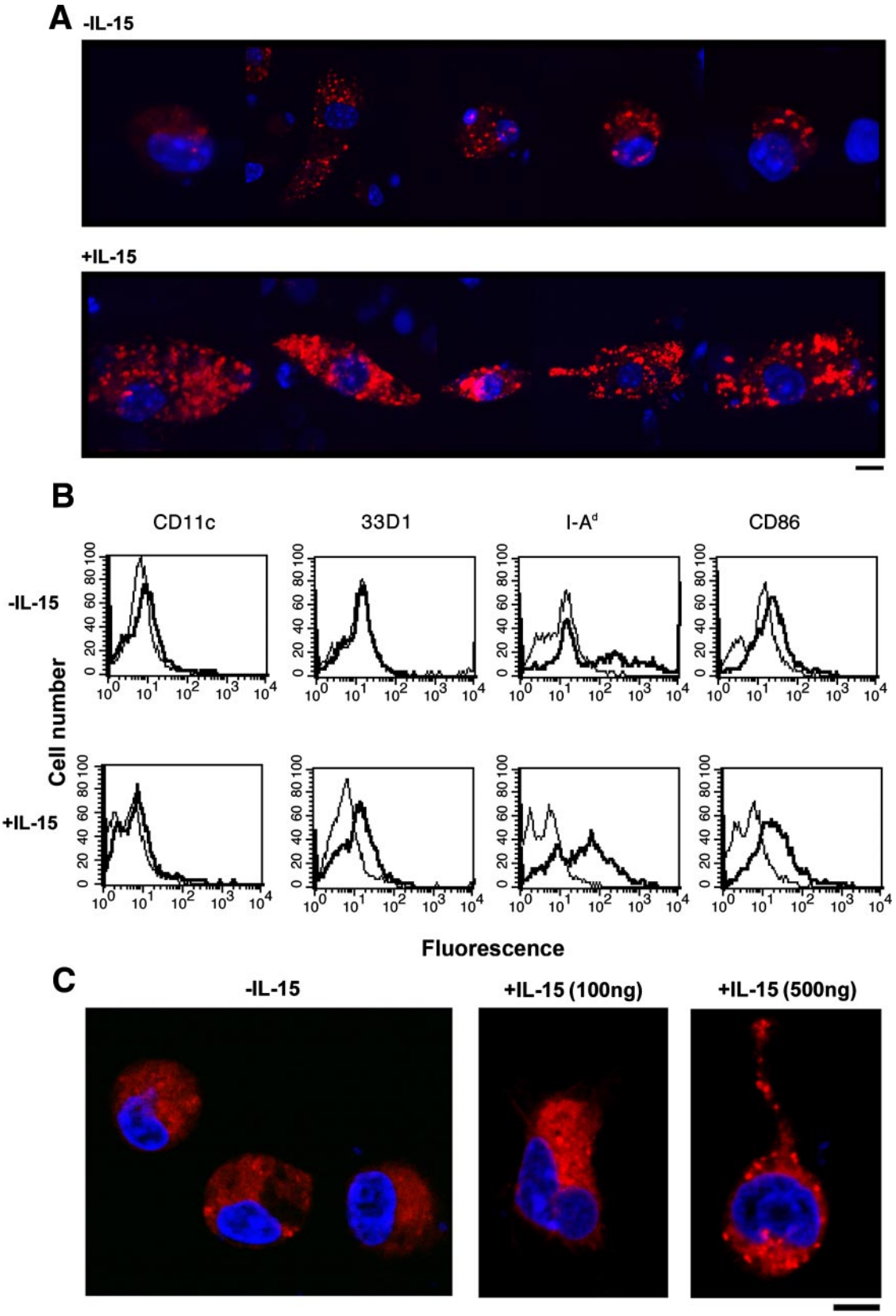

FIG. 1. Expansion and condensation of lysosomes in mouse monocytes treated in culture with IL-15. Spleen cells isolated from normal BALB/c mice were cultured with IL-15 (100 ng/ml) for 48 h. LysoTracker and Hoechst dye were added to the culture, and cells were examined for morphological changes. (A) IL-15 induced many acidic organelles (red) in spleen cells with an extended, DC-like shape as shown in the composite image in this panel. (B) In vitro spleen cells cultured with IL-15 induced greater cell surface expression of 33D1, I-A^d, and CD86 than spleen cells cultured without IL-15. Thin lines in the histograms represent staining with isotype-matched control antibodies, while thick lines represent staining with the indicated antibodies. (C) CD11c-positive cells cultured with IL-15 acquired a mature-DC shape. Bars in panels A and C, 5 μm.

PeproTech, Inc. (Rocky Hill, NJ). Solvent-extracted lipopolysaccharide (LPS) was obtained from InvivoGen (San Diego, CA). LysoTracker and Hoechst dye were obtained from Molecular Probes (Eugene, OR). Rat anti-mouse DC (clone 33D1) marker (monoclonal antibody 33D1-biotin; rat immunoglobulin G2b [IgG2b]) was purchased from Leinco Technologies, Inc. (St. Louis, MO). Goat anti-rat IgG fluorescein isothiocyanate (FITC)-labeled streptavidin was purchased from Becton Dickinson (BD) Pharmingen (San Diego, CA). FITC-labeled CD11c, I-A^d, and CD86 were also purchased from BD Pharmingen. Isotyping kits for mouse antibody were purchased from Bio-Rad (Hercules, CA).

Vaccinations. Pathogen-free male BALB/c mice, 6 to 8 weeks old, were obtained from Charles River Laboratories (NCI—Frederick, Frederick, MD). The mice were maintained under pathogen-free conditions and fed laboratory chow and water ad libitum. For measuring antibody responses, mice were inoculated with 10 μg of STEBVax on days 0, 14, and 28 either without an adjuvant, with the adjuvant alone, or with the adjuvant and IL-15 (0.1 μg, 0.5 μg, or 5 μg/mouse) in phosphate-buffered saline (PBS). The STEBVax was formulated (i) in 50 mM glycine, 0.9% NaCl at pH 8.3 either without the adjuvant or with the Good Manufacturing Practice aluminum hydroxide adjuvant alhydrogel (Superfos Biosector, Kvistgaard, Denmark) at a 7:1 (wt/wt) ratio of aluminum to antigen or (ii) with 70 μg of solvent-extracted LPS (InvivoGen, San Diego, CA). Injections (100 μl, intramuscular) were given in the upper thigh on each hind leg by using a standard 1-ml syringe with a 30-gauge needle (BD, Franklin Lakes, NJ). Blood was collected from the tail vein on days 10, 24, and 38 for serum antibody determinations.

SEB challenge. The mice were challenged intraperitoneally (i.p.) on day 42 with 60 50% lethal doses (LD_{50}) or 10 LD_{50} of wild-type SEB (DSTL, Salisbury, United Kingdom), followed 4 h later with a potentiating i.p. injection of 70 μg LPS (200 μl of PBS containing 70 μg of LPS/mouse) from *Escherichia coli* type 055:B5 (Difco, Detroit, MI), as described previously (32). Survival was recorded for 7 days after the challenge. Challenge controls were age-matched mice injected with either LPS or SEB only.

Antibody determinations. Titers of serum antibodies were determined by enzyme-linked immunosorbent assay (ELISA). Immulon 2HB 96-well plates (MTX Lab Systems, Vienna, VA) were coated with 2 μg/ml of STEBVax in PBS (pH 7.4) and incubated for 1.5 h at 37°C. The plates were washed three times with 0.1% Tween 20 in PBS and blocked with 0.2% (wt/vol) casein in PBS (1.5 h at 37°C). After three washes, serially diluted serum samples in 0.02% casein-PBS were incubated for 1.5 h at 37°C. The plates were washed again three times, goat anti-mouse IgG (Sigma, St. Louis, MO) conjugated to horseradish peroxidase was added at a 1:15,000 dilution in 0.02% casein-PBS, and the plates were incubated for 1.5 h at 37°C. After a wash, the plates were developed for 30 min at room temperature with 3,3′,5,5′-tetramethylbenzidine and the absorbances determined at 650 nm.

For determining antibody isotypes in serum, an ELISA was performed by using the Bio-Rad isotyping kit according to the manufacturer's protocol. Briefly, antigen (STEBVax)-coated wells were allowed to react with mouse antiserum and then with appropriate rabbit anti-mouse antibody isotypes. Immunoglobulin class types were then determined with goat anti-rabbit (H+L)-horseradish peroxidase conjugate. Serum antibody titers were determined as reciprocal endpoint dilutions (means ± standard errors of the means [SEM]) from a linear plot (i.e., the dilution required for readings to reach values corresponding to absorbance at the background of about 0.028).

Spleen cell isolation and purification of CD11c. Spleens were removed aseptically from euthanized mice. Single-cell suspensions were prepared by lysing red blood cells by using ammonium chloride-potassium lysing buffer (Cambrex, Walkersville, MD), followed by several washings. Spleen cells were then cultured with or without IL-15. For isolating CD11c-positive cells, spleen cells were suspended (10^8 cells/400 μl) in cold PBS supplemented with 2 mM EDTA and 0.5% bovine serum albumin (fraction V; Sigma-Aldrich, St. Louis, MO). Paramagnetic beads coated with anti-CD11c (Miltenyi Biotec, Auburn, CA) were mixed with spleen cells (10^8 cells/100 μl). The antibody-labeled cells were incubated for 15 min (4°C), washed, and passed through a type LS or MS iron fiber column placed within a strong magnetic field (Miltenyi Biotec). CD11c-positive cells bound to the column were eluted with buffer. Spleen cells or selected CD11c cells were cultured with IL-15 in RPMI 1640 medium containing L-glutamine and 5% fetal bovine serum.

Flow cytometry. To examine cell surface expression of proteins on mouse spleen cells, the cells were incubated (20 min, 4°C) with FcR blocking reagent (Miltenyi Biotech Inc.), washed twice with Hanks' balanced salt solution containing 1% bovine serum albumin, and then incubated (30 min, 4°C) with FITC-labeled or phycoerythrin (PE)-labeled control or isotype-matched monoclonal antibodies. For unlabeled monoclonal antibodies (detecting 33D1, CD86, CD40, and I-A^d), cells were first incubated (30 min, 4°C) with primary antibody, washed briefly by centrifugation, and then incubated with goat anti-mouse IgG (FITC or PE conjugated). Unbound antibody was removed by washing the cells with Hanks' balanced salt solution (4°C) and centrifugation. After two additional washes, the labeled cells were fixed with 1% paraformaldehyde in PBS, and the cell-associated immunofluorescence was measured by flow cytometry (FACScan; Becton Dickinson, San Jose, CA).

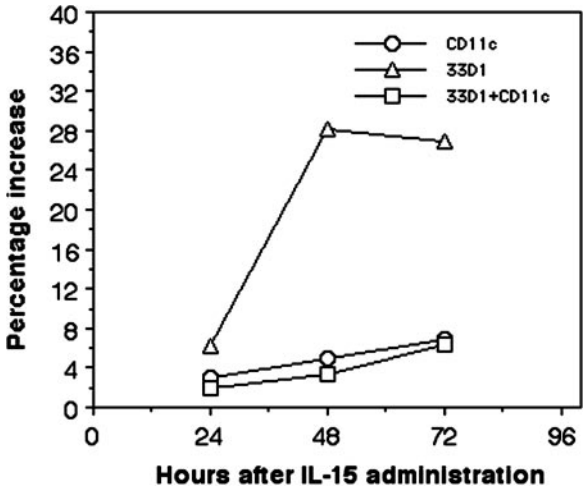

FIG. 2. Administration of IL-15-induced $CD11c^+$, $33D1^+$, and both $CD11c^+$ and $33D1^+$ DC in mouse spleen. BALB/c mice were injected with IL-15 (0.5 μg/mouse, i.p.); spleens were collected at 24 h, 48 h, and 72 h; and spleen cells were labeled with FITC-conjugated antibody to CD11c or 33D1 and then with FITC-labeled secondary antibody or isotype-matched control antibody. Cells positive for CD11c, 33D1, and both CD11c and 33D1 were detected by flow cytometry through the acquisition of 10,000 events from the labeled cell populations. Data are presented as percentages of increase by subtracting the values for untreated controls from each data point. Results showed that IL-15 administration increased the numbers of cells positive for CD11c and 33D1, as well as for CD11c and 33D1, in the spleen. A chi-square test of statistical analysis comparing the proportions of positive cells either within each time point or within each group showed a significant difference in the proportions of positive cells between the IL-15-treated group and the untreated group ($P \leq 0.005$).

RESULTS

Mouse spleen cells cultured with IL-15 acquire characteristics of mature DC. Our previous results showed that human monocytes are transformed by IL-15 into mature DC that stimulate antigen-specific T-cell responses in vitro (29). We first examined whether mouse IL-15 would exert a similar effect on mouse monocytes in culture. Monocytes were isolated from spleen cells by plastic adherence and treated with IL-15. We observed by LysoTracker staining and confocal microscopy that the majority of IL-15-treated cells in culture appeared large and with extended shapes and contained many acidic organelles compared to untreated controls (Fig. 1A). We also noticed that IL-15-treated monocytic cells possessed a modest increase in the cell surface expression of I-A^d, CD86, and 33D1 compared to that in untreated controls (Fig. 1B). These preliminary results confirmed that spleen cells acquired a DC-like phenotype when cultured with IL-15 and prompted us to examine CD11c-positive cells. Purified CD11c-positive cells, when cultured with IL-15, similarly underwent morphological changes resulting in mature DC (Fig. 1C), and no change in the

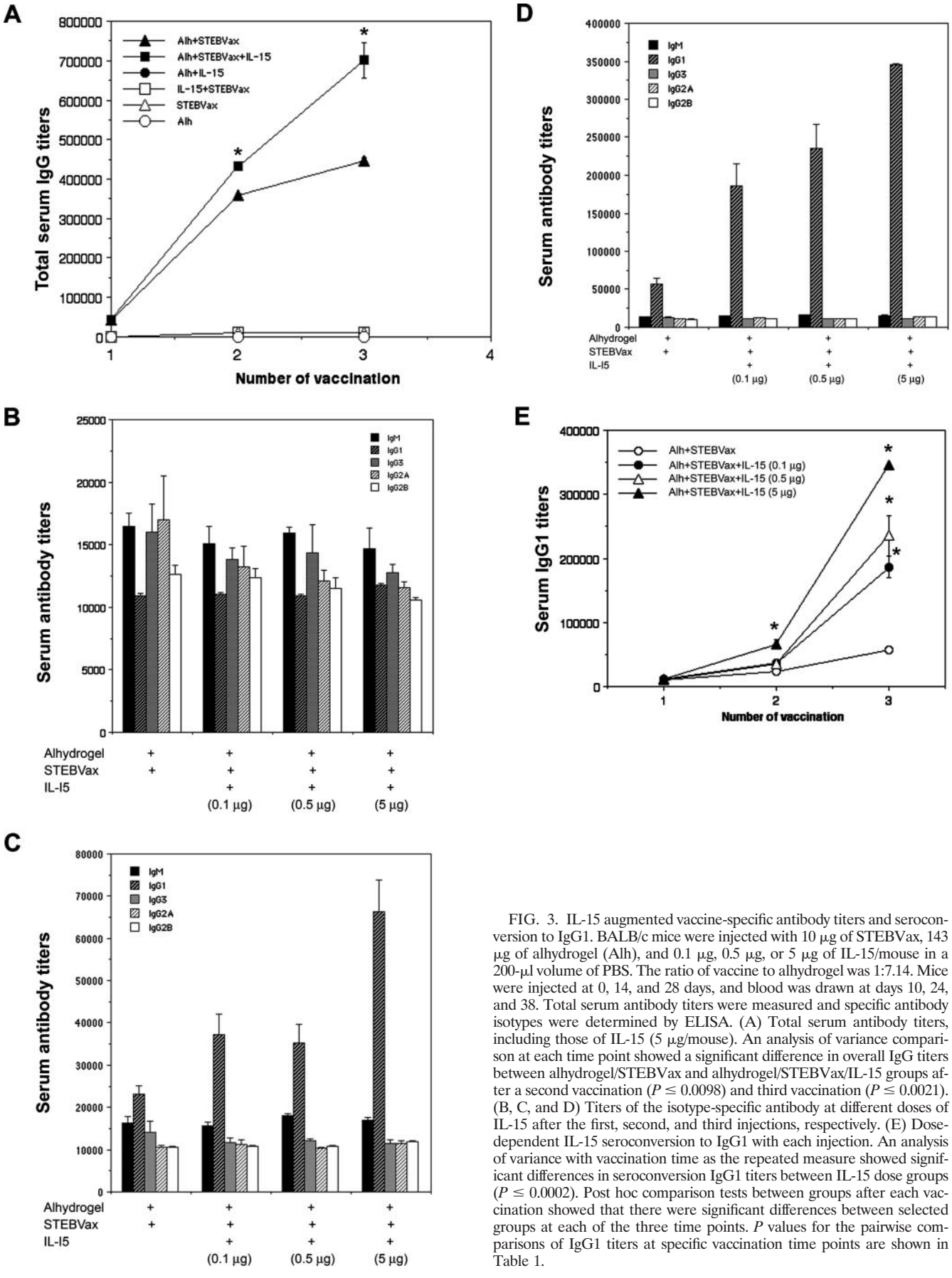

FIG. 3. IL-15 augmented vaccine-specific antibody titers and seroconversion to IgG1. BALB/c mice were injected with 10 μg of STEBVax, 143 μg of alhydrogel (Alh), and 0.1 μg, 0.5 μg, or 5 μg of IL-15/mouse in a 200-μl volume of PBS. The ratio of vaccine to alhydrogel was 1:7.14. Mice were injected at 0, 14, and 28 days, and blood was drawn at days 10, 24, and 38. Total serum antibody titers were measured and specific antibody isotypes were determined by ELISA. (A) Total serum antibody titers, including those of IL-15 (5 μg/mouse). An analysis of variance comparison at each time point showed a significant difference in overall IgG titers between alhydrogel/STEBVax and alhydrogel/STEBVax/IL-15 groups after a second vaccination ($P \leq 0.0098$) and third vaccination ($P \leq 0.0021$). (B, C, and D) Titers of the isotype-specific antibody at different doses of IL-15 after the first, second, and third injections, respectively. (E) Dose-dependent IL-15 seroconversion to IgG1 with each injection. An analysis of variance with vaccination time as the repeated measure showed significant differences in seroconversion IgG1 titers between IL-15 dose groups ($P \leq 0.0002$). Post hoc comparison tests between groups after each vaccination showed that there were significant differences between selected groups at each of the three time points. *P* values for the pairwise comparisons of IgG1 titers at specific vaccination time points are shown in Table 1.

expression of 33D1 and CD11c was observed in culture (data not shown). These results suggest that IL-15 has an effect on the maturation of spleen DC. It is speculated that the endogenous availability of IL-15 may be effective in inducing the DC maturation that led us to examine the in vivo effect of IL-15 in mice.

Administration of IL-15 to mice increased the maturation of DC and expression of cell surface costimulatory molecules. Next we examined the endogenous effect of IL-15 on the maturation of DC in mouse spleen cells. For this, spleen cells were isolated from IL-15-treated mice and examined for their DC number by analyzing the cell surface expression markers CD11c and 33D1 (26). Increase in the numbers of cells positive for CD11c and 33D1, as well as for CD11c and 33D1 (doubly positive cells), were detected in total spleen cell populations (Fig. 2) in IL-15-treated mice compared to the numbers in the control (no IL-15 treatment). These CD11c- and 33D1-positive spleen DC also showed an increase in the expression of I-A^d and CD86 (data not shown). In addition, we examined the effect of IL-15 on spleen DC when it was used in conjunction with the vaccine STEBVax (38) and an adjuvant (alhydrogel) used in humans. In vivo administration of recombinant IL-15 with STEBVax and alhydrogel increased the number of CD11c-positive cells expressing costimulatory molecules in mouse spleen cells compared to that in saline-treated controls (data not shown). These results suggest that the intramuscular injection into mice of IL-15 either alone or in the presence of a vaccine and an adjuvant resulted in more mature $CD11c^+$ DC from the spleen.

Inclusion of IL-15 to STEBVax and alhydrogel increased STEBVax-specific antibody titers and seroconversion to IgG1. Earlier results from our laboratory demonstrated that STEBVax administered with alhydrogel protected mice from a lethal dose of SEB toxin by stimulating neutralizing antibody production (38). To examine the capacity of IL-15 to serve as a DC activator of antibody responses to STEBVax, we incorporated IL-15 in an alhydrogel-based STEBVax vaccination regimen. Our results show that including IL-15 with STEBVax increased serum antibody titers with each vaccination gradually compared to results with STEBVax and alhydrogel alone (Fig. 3A). While no change in antibody isotype profile was observed after the first dose in either the presence or absence of IL-15 (Fig. 3A), a second or third dose significantly shifted the response toward an IgG1 isotype with the inclusion of IL-15 (Fig. 3B and C). This predominant isotype class switching to IgG1 and the increase in IgG1 titers were concurrent with the increase in total antibody titers (compare Fig. 3B with Fig. 3C and D). Furthermore, increased antibody titers to STEBVax in the presence of IL-15 progressively led to a Th2-type response, with predominant antibody class switching toward IgG1 occurring in a dose-dependent manner along with the number of vaccinations (Fig. 3E). These results suggested that increased Th2-type responses to STEBVax were likely attributed to IL-15 by activation of DC because the endogenous administration of IL-15 increased $CD11c^+$ mature DC in mice.

Coadministration of IL-15 with STEBVax and alhydrogel increased protection from a lethal SEB challenge. To confirm that increased antibody titers correlated with protective immunity, we measured the survival of mice following toxic shock syndrome induced by SEB (32). After the three vaccinations with 20 μg of STEBVax, formulated with alhydrogel and IL-15, mice were challenged at 42 days with 60 LD_{50} of SEB plus LPS to potentiate toxicity (32). We observed 80% survival from toxic shock for mice immunized with or without IL-15 (Fig. 4A), and all control mice that were challenged with either LPS or SEB alone survived. To demonstrate increased efficacy with IL-15, we lowered the amount of vaccine administered. Mice received three vaccinations with 5 μg of STEBVax-formulated alhydrogel with IL-15 and were then challenged with 10 LD_{50} of SEB (plus LPS). In comparison to mice vaccinated with STEBVax plus alhydrogel, mice that were vaccinated with STEBVax, alhydrogel, and IL-15 showed a marked increase in protection and a longer survival time from lethal toxic shock (Fig. 4B). These results confirmed that IL-15 enhanced both antibody seroconversion to a Th2-type response to STEBVax and protection from lethal toxic shock.

TABLE 1. *P* values for the pairwise comparisons of IgG1 titers at specific vaccination time points

Concn of IL-15 (μg/mouse) used in comparison	*P* value for IgG1 titers at vaccination time[a]:		
	1	2	3
0 vs 0.1	0.5253	**0.0044**	**0.0479**
0 vs 0.5	0.9888	**0.0093**	**0.0038**
0 vs 5	**0.0006**	<0.0001	**0.0055**
0.1 vs 0.5	0.6978	0.9266	0.4388
0.1 vs 5	**0.0029**	**0.0014**	0.6028
0.5 vs 5	**0.0009**	**0.0007**	0.9836

[a] Values in boldface type are significant. Time points 1, 2, and 3 correspond to 0, 14, and 28 days, respectively.

DISCUSSION

The induction of long-lasting cellular immunity by IL-15 has been studied extensively (20, 23). However, our results suggest that IL-15 also promotes antibody responses through a mechanism linked to DC maturation. The inclusion of recombinant IL-15 in an alhydrogel-based vaccination regimen induced the maturation of DC and augmented vaccine-specific protective immunity (seroconversion to IgG1) in a lethal SEB challenge. The adjuvant-like effects of IL-15 may influence antibody responses at several levels. For example, IL-15 enhances proliferation and the immunoglobulin secretion of human peripheral B cells (1, 2, 11), inhibits apoptosis induced by anti-IgM (3), and induces the proliferation of malignant B cells (34). Membrane-bound IL-15 significantly modulated GC B-cell reactions (25). The presence of a positive-feedback mechanism between GC B cells and FDC is plausible because IL-15 reportedly increases costimulatory molecules on DC by means of an autocrine loop (28, 35).

The IL-15 mRNA has been found in a variety of cell types (9). However, IL-15 protein is secreted at such low levels that it is not detectable in the serum under physiological conditions. It was suggested that the induction of IL-15 and the IL-15 receptor establishes an autocrine loop that is essential for the survival of DC (28, 17). Our results show that the endogenous administration of IL-15 increased the expression of costimulatory molecules on spleen DC and that this increased expression influences vaccine-specific antibody conversion to IgG1 in the

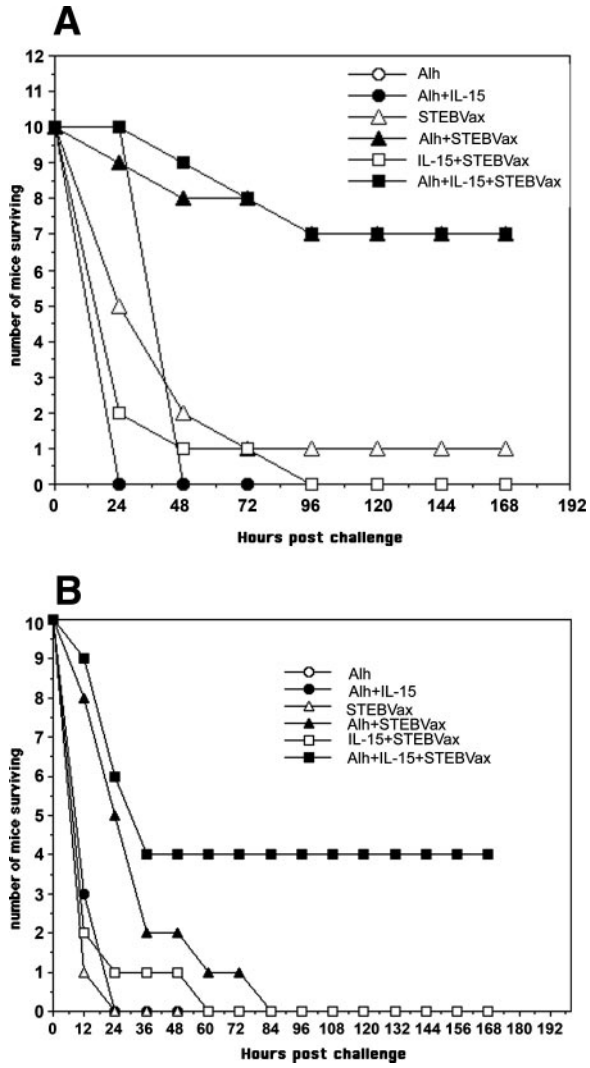

FIG. 4. Coadministration of IL-15 with STEBVax and alhydrogel (Alh) increased the protection of mice from lethal SEB toxin challenge. (A) BALB/c mice were vaccinated with 20 μg of the vaccine (STEBVax), 143 μg of alhydrogel, and 0.1 μg of IL-15/mouse in a 200-μl volume of PBS. Mice were injected at 0, 15, and 30 days and then challenged first with SEB (60 LD_{50}) and then with LPS (70 μg/mouse) 4 h later. Survival was recorded for 7 days after the challenge. (B) BALB/c mice were vaccinated with 5 μg of the vaccine, 143 μg of alhydrogel, and 0.1 μg of IL-15/mouse in a 200-μl volume of PBS. Mice were injected at 0, 15, and 30 days and then challenged first with SEB (10 LD_{50}) and then with LPS (70 μg/mouse) 4 h later. Survival was recorded for 7 days after challenge by monitoring every 12 h. Kaplan-Meier survival analysis showed that the alhydrogel/STEBVax group had median and mean survival times of 30 h and 34.8 h (SEM, 7.0) compared to those of the alhydrogel/STEBVax/IL-15 group, which had median and mean survival times of 36 and 82.8 (SEM, 24.2), respectively. Comparison of survival curves between the alhydrogel/STEBVax and alhhydrogel/STEBVax/IL-15 group showed a P value of 0.0877. Challenge controls were age-matched mice injected with either LPS or SEB only. There were no deaths in any of the challenge control groups (SEB or LPS only).

presence of alhydrogel, an aluminum hydroxide adjuvant. Earlier reports indicated that aluminum hydroxide stimulates the increased expression of major histocompatibility complex class II costimulatory molecules on monocytes and proinflammatory cytokines that activate T cells (37). Further, activated Th2 cells release IL-4, which suppresses macrophage growth, increasing the expression of major histocompatibility complex class II and costimulatory molecules that characteristically lead to the enhanced antigen-presenting function of DC. Our results could also be explained by a synergistic effect between IL-15 and alhydrogel, which directly affects B-cell activation and leads to enhanced vaccine-specific IgG1 responses. B cells generally respond to vaccination or infection by diversifying the antibodies that they produce through two processes: antibody class switching through recombination and somatic hypermutation of their antigen-binding regions. Somatic hypermutation, in conjunction with B-cell selection, results in the increased antigen-binding ability of an antibody. It is interesting that the secretion of class-switched antibodies requires additional stimulation upon B-cell antigen receptor engagement and exposure to IL-15 (14).

In the current study, our results showed IL-15 enhancement of STEBVax-specific seroconversion predominantly to IgG1, which is indicative of the Th2-type response. Classical adjuvants were presumed to activate adaptive immune responses by Toll-like receptors (TLRs) (8). However, very recent reports challenge the notion that TLRs are not the essential link between the innate and adaptive immune systems where classical adjuvants are concerned (8). Nevertheless, adjuvants or immunostimulators have important implications as vaccine additives. Therefore, immunomodulators that have functions other than that of TLRs and contribute to the enhancement of protective antibody responses to vaccines would be highly desirable. In this study, our results identified the immunostimulatory function of IL-15 that enhanced vaccine-specific antibody responses. This raises the possibility that IL-15 may serve as a potent immunomodulator that broadens immunity by augmenting responses of humoral, as well as cellular, immunity.

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Research was conducted in compliance with the Animal Welfare Act (39a) and other federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council (20a). The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Views expressed in this paper are ours and do not purport to reflect official policy of the U.S. Government.

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